PROJECT NUMBER: 1730
PROJECT TITLE: Plant Cell & Tissue Culture Research
PROJECT LEADER: I. L. Uydess
PERIOD COVERED: February, 1988

I. TOBACCO IDENTICAL PRESERVATIVES

A. **Objective**: To develop procedures and to establish microbiological screens for the evaluation of new, nature-identical preservatives as replacements for and/or as adjuncts to propylparaben.

B. **Status & Results**:

1. Antimicrobial/Preservative Screens:

a. Phase I Screens: Several aqueous phase fractions of oriental tobacco (grade LSB) and one sucrose ester fraction from samsun tobacco (D. Danehower, N.C.State University) were tested in a scaled-down version of the agar inclusion assay. The aqueous fractions were found to have marginal activity at about 5000 µg/ml, while the sucrose ester fraction (92% pure) was observed to have substantial activity at or below 500 µg/ml. A similar result to this was reported previously for a less-well purified sample of sucrose esters isolated from oriental tobacco (grade LSB, D. Teng, see January, 1988 monthly).

2. Microbiological Investigations:

a. Phase II Screen: Additional samples of sterile, Park 500 SEL have been induced to spoil (increase in pH, cell number, and odor) under controlled laboratory conditions in support of the development of a standard operating procedure for a Phase II screen. In each case, fresh samples of autoclaved SEL were inoculated with the mixed culture of bacteria obtained from a previously spoiled sample of SEL. After multiple serial passages, four gram-positive bacteria continued to comprise the mixed culture, although the relative ratios of each has been observed to vary with time and increasing pH as spoilage of the SEL progressed. Two of the organisms appeared to predominate at more acidic pHs (pH 5.0 - 6.5), while the other two predominated at the more neutral and basic pHs (pH 7.0 - 8.5). Similar attempts to employ each of the isolates alone have proven unsuccessful. In each case, the individual isolates have not been able to grow successfully in sterile SEL (three die off, one grows marginally).

A preliminary Phase II control screen was successfully run in sterile SEL using the mixed culture exposed to various levels of propylparaben in PG. After 48 hrs, the cell counts (CFUs) in the samples exposed to 300, 500 and 1000 µg/ml PP-PG were found to be 3.5 logs lower than that of the

2022162316

Source: https://www.industrydocuments.ucsf.edu/docs/nyxm0000

control flasks (approximately, 1.0 E4 vs 5.0 E7, respectively). In addition, the relative sensitivity of each of these isolates to PP-PG was evaluated in the Phase I, agar-inclusion assay. Two of the organisms were found to be less sensitive to PP-PG than the others in this assay system.

b. Biochemical Investigations: Chemical evaluations of the chemistry of spoilage are also being conducted in collaboration with members of the Analytical Research Division. Recently, ^{13}C-NMR (Dr. Jan Wooten) has proven to be very effective in following the time course of chemical change in samples of laboratory spoiled SEL. This information will be used to help establish biochemical criteria for the spoilage of SEL in support of the overall goals of this program.

c. Adhesive Spoilage Study: Samples of a starch-based adhesive have been received from B. Semp for the investigation of spoilage. Multiple aliquots of this material are being prepared so that they can be exposed to the air to promote contamination.

C. **Conclusions**:

1. Sucrose esters continue to exhibit substantial antimicrobial activity in the Phase I screens.

2. Laboratory induced spoilage in SEL appears to be the product of the metabolic activity of one or more gram-positive bacteria which may have complementary (or interdependent) metabolic requirements. Additionally, the progression of spoilage is paralleled by a characteristic shift in the relative composition of the mixed culture as the pH of the SEL rises.

D. **Plans (March, 1988)**:

1. Additional samples of the sucrose ester fractions from both samsun and LSB oriental tobaccos will be tested in the Phase I, agar inclusion screen.

2. Further analysis of the biochemical and microbiological characteristics of spoilage will be conducted in support of the development of a Phase II, SEL preservative screen.

3. The spoilage of a starch-based adhesive (provided by B. Semp) will be investigated. Propylparaben in PG will be evaluated as a possible preservative for this material.

2022162317

Source: https://www.industrydocuments.ucsf.edu/docs/nyxm0000